

## LOOKING FOR SYNERGISTIC EFFECTS OF CHEMICAL DISINFECTANTS AND UV FOR A MORE EFFECTIVE WATER TREATMENT

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### Abstract

The main objective of this study is to explore possible synergistic or additive effects of combinations of chemical disinfectants (sodium hypochlorite, peracetic acid, hydrogen peroxide, chlorine dioxide) and UV in their efficacy in inactivating free-living bacteria and removing biofilms. In contrast to most studies, this study examines disinfection of municipal water in a pilot-scale system using a mixed bacterial suspension, which enables a better simulation of the conditions encountered in actual industrial environments. It was shown that the combination of either hypochlorite, hydrogen peroxide, peracetic acid, or chlorine dioxide with UV yielded additive effects on the inactivation of free-living bacteria. Actual synergy was observed for the combination of UV and 5 ppm hydrogen peroxide. Regarding biofilm treatment, additive effects were observed using the combination of hydrogen peroxide and UV. The promising results obtained in this study, indicate that the combination of UV and chemical disinfectants can considerably reduce the amount of chemicals required for the effective disinfection and treatment of biofilms.

### Keywords:

Biofilm; Chlorination; Disinfection; Hydrogen peroxide; Peracetic acid; pilot-scale system UV-treatment;

## Introduction

In both industrial and non-industrial applications, water is circulated and reused. However, continuous water reuse leads to a considerable decrease in water quality due to the proliferation of microorganisms and biofouling. Biofilm formation frequently results in operational problems, such as increased fluid frictional resistance, reduced heat transfer in heat exchangers, and unexpected corrosion of stainless steel (Ludensky, 2003; Coetser and Cloete, 2005). Moreover, biofilms are difficult to remove because they provide a niche where resident microbes are protected against environmental stress or disinfectants. Furthermore, biofilms may be regarded as reservoirs of potential pathogens, because they release potential pathogens in the planktonic environment on a rather constant basis (Lehtola et al., 2007). So it is clear that biofilm formation can have a considerable economic impact and pose a threat to human health as well. Hence, an effective, cost-efficient, and environmental-friendly water treatment is required to reduce biofilm-associated problems.

Currently, a variety of water disinfection techniques are commonly used, including biocide application and ultraviolet light (UV) (Pozos et al., 2004). UV penetrates the bacterial cell membrane and blocks DNA replication, which eventually results in lethal damage. Disadvantages of UV treatment are its limited efficacy in highly light scattering or absorbing solutions, and possible photoreactivation or dark repair of UV-damaged microorganisms, enabling regrowth of the microbial population under certain conditions (Jungfer et al., 2007). For many years the preferred biocide has been hypochlorite, because of its effectiveness, ease of use, and relatively low cost (Grant and Bott, 2005). However, the use of hypochlorite is increasingly debated because of the formation of toxic, mutagenic and/or carcinogenic disinfection by-products, such as trihalomethanes, haloacetic acids, and chlorine residuals (Gopal et al., 2007). Environmental concern has thus led to the awareness that the use of toxic biocides, like hypochlorite, should be replaced or reduced. This can be achieved either by (i) finding an alternative biocide that does not generate toxic by-products; or by (ii) reducing the hypochlorite concentration. Chlorine dioxide, with its strong oxidation capacity and its high effectiveness against a wide variety of microorganisms, is often used as an alternative to hypochlorite because it doesn't produce significant amounts of chlorinated toxic by-products (Gomez-Lopez et al., 2009). Hydrogen peroxide and peracetic acid (PAA) are alternative biocides that do not generate significant amounts of toxic or mutagenic by-products, or chemical residues in effluents (De Luca et al., 2008).

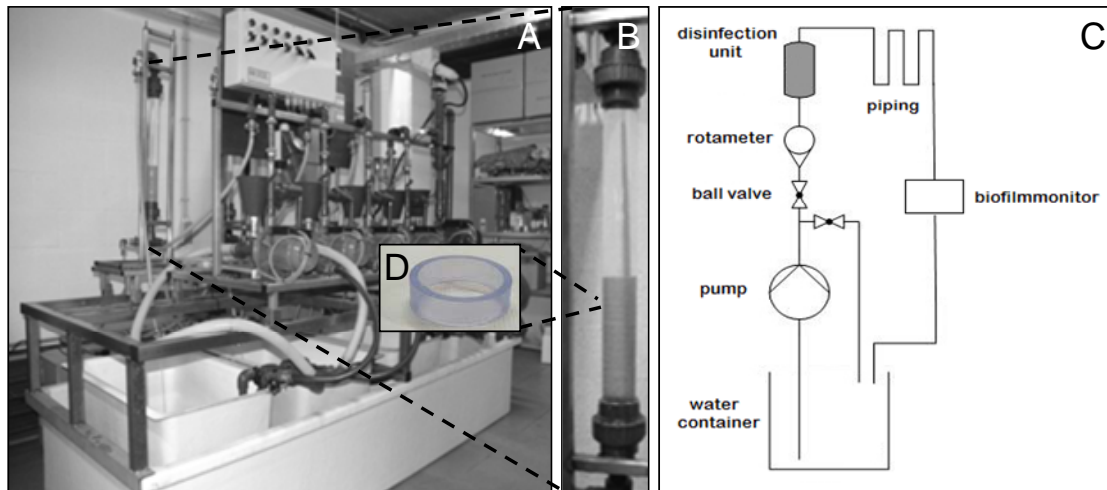
Apart from using biocides that are less hazardous for the environment, the reduction of toxic by-products may be achieved by combining hypochlorite treatment with a physical disinfection technique, such as UV irradiation. Therefore, the main objective of this study was to explore possible synergistic or additive effects of UV and chemical disinfectants on the inactivation of free-living bacteria and treatment of biofilms in a pilot-scale system.

## Materials and Methods

### *Design of the pilot-scale system*

The pilot-scale system (Fig. 1) consists of 4 identical subsystems, each containing a water container (up to 160 l), provided with a pump (adjustable water flow of 60 to 1000 l/h) to circulate the water through a piping system (20 m), a disinfection unit and a biofilm device. In this way, four independent experiments, including a control (i.e. an untreated water system), can be carried out simultaneously. The biofilm device consists of an outer tube, in which 30

polycarbonate rings, with an inner diameter of 3.3 cm and an inner surface of 11.5 cm<sup>2</sup>, are placed. Biofilms can develop on the inner surface of these rings. During the experiments, the water was pumped (at 180 l/h) from the water container, through the disinfection unit, the piping system and the biofilm device, after which it was collected again in the water container. Temperature and pH were monitored throughout the experiment and kept at 30°C and pH 7, respectively.



**Figure 1.** A photo of the pilot-scale system (A), and a close-up of the biofilm device used in this study, containing polycarbonate rings (B). A schematic overview of a single subsystem is presented in C (adopted from Hulsmans et al., 2010). A polycarbonate ring is presented in more detail (D).

#### *Disinfection treatments*

Different concentrations of 4 disinfectants (sodium hypochlorite, PAA, hydrogen peroxide, and chlorine dioxide) were evaluated. The desired hypochlorite concentration was attained as described in Vankerckhoven et al. (2010 and 2011). Active chlorine concentrations were monitored twice a day according to the *N,N*-diethyl-*p*-phenylenediamine (DPD) colorimetric method. Chlorine dioxide was generated according to the TwinOxide® system. According to the recommendations of the manufacturer, chlorine dioxide concentrations were monitored by means of iodometric titrations. Hydrogen peroxide levels were obtained by adding the appropriate dose of a silver-stabilized 597 g/l hydrogen peroxide stock solution (Delgo-San TR-50-SL). Disinfection dosages of PAA were achieved by adding the correct amount of a stock solution containing 5% PAA, 22% hydrogen peroxide and 15% acetic acid (Peraclean®). Concentrations of PAA and hydrogen peroxide were monitored using titrations with potassium permanganate. UV irradiation was applied continuously using a flow-through system with a low pressure mercury arc source (peak emission at 254 nm; UV-fluence 234 mJ/cm<sup>2</sup>) enclosed in a tubular chamber. Each disinfection condition was independently repeated at least twice.

#### *Bacterial strains, growth conditions, inoculation, and biofilm formation*

The process water (100 l municipal water) was inoculated with a mixed bacterial suspension, containing *Escherichia coli* (strain LMG 2092T), *Pseudomonas aeruginosa* (strain LMG 1242T), *Flavobacterium breve* (strain LMG 4011T) and *Aeromonas hydrophila* (strain LMG 2844T). The bacteria used in this study were selected because they are able to grow in water distribution systems and/or because they are frequently found in drinking-water biofilms (Szewzyk et al.,

2000). Process water was inoculated to achieve, for each strain, a concentration of approximately  $10^6$  colony forming units (CFU)/ml. Subsequently, the water was circulated through the pilot-scale system. Prior to commencing disinfection, biofilm was allowed to develop on the polycarbonate rings in the biofilm device for 65 h. After this period, the first samples (at 0 h) were taken as described below and the disinfection was initiated. Samples were taken every 24 h to monitor the level of free-living and biofilm-associated bacteria. A control (i.e. an untreated water system) was included in all experiments.

#### *Quantification of free-living and biofilm-associated bacteria*

For each independent experiment, samples were taken from the water containers in triplicate every 24 h in order to assess the number of free-living bacteria. The bacterial level was determined by traditional culture-plate enumeration on Brain Heart Infusion Agar. To quantify biofilms, polycarbonate rings were removed from the biofilm device in triplicate for each sampling point. The rings were briefly washed with sterile saline solution to remove the non-biofilm cells. Subsequently, the biofilm was scraped from the rings and resuspended in 10 ml of sterile saline solution. The number of resuspended bacteria was enumerated by culture-plate enumeration as described above, and the amount of bacteria residing in the biofilm (expressed in CFU/cm<sup>2</sup>) was determined.

Bacterial levels were log transformed before statistical analysis. Significant differences between results were determined using Student t-tests.

## **Results and Discussion**

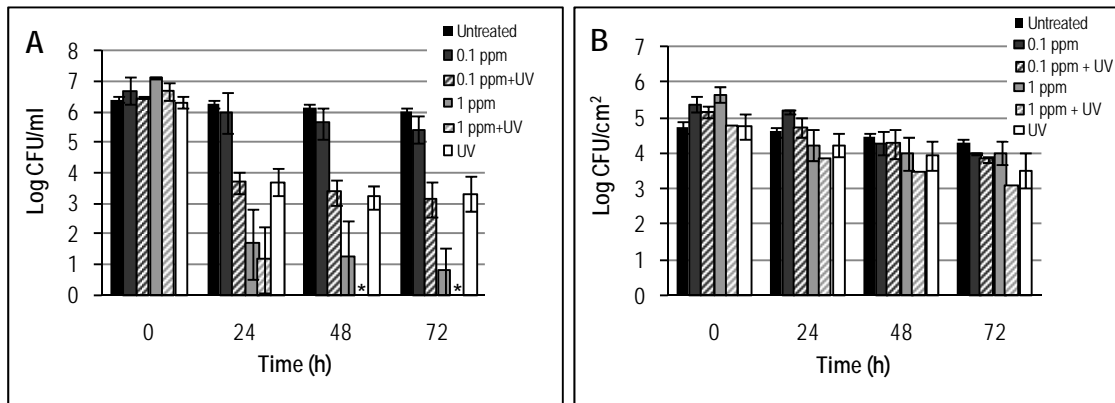
In this study, pilot-scale experiments were used to compare 5 different disinfection techniques (hypochlorite, chlorine dioxide, PAA, hydrogen peroxide, and UV irradiation) for their disinfection efficacy. The disinfection efficacy was evaluated by assessing two parameters at regular time intervals: (i) the number of bacteria in suspension (free-living bacteria); and (ii) the number of bacteria attached to the polycarbonate rings in the biofilm device (biofilm-associated bacteria). In addition, the disinfection efficacy of the respective combinations with UV was also assessed to reveal possible additive or synergistic effects. The process is called additive when the combination of disinfection techniques produce an effect that is greater than the separately measured individual effects. Synergism is described as “the combination of techniques that is more effective than is expected from the single component effectiveness of its constituents” (Dykstra et al., 2007). In the following, hypochlorite, chlorine dioxide, hydrogen peroxide, and PAA, as well as their respective combinations with UV were evaluated for their disinfection efficacy.

#### *Treatment with hypochlorite and UV irradiation*

Treatment with UV resulted in a significant decrease in the number of free-living bacteria (Fig. 2A). After 72 h of UV treatment, a reduction of 3.0 log CFU/ml was achieved. The largest reduction was observed after 24 h. From thereon, the bacterial level remained more or less constant. In the pilot plant study of Hassen et al. (2000) a 2.7 log reduction in fecal coliforms was achieved in waste water using UV- irradiation with a slightly lower fluence (162 mJ/cm<sup>2</sup>).

UV treatment was considerably less effective in killing biofilm-associated bacteria and only resulted in a 1.2 log CFU/cm<sup>2</sup> reduction after 72h (Fig. 2B). This is because the biofilm is not in direct contact with the UV source, and is in agreement with Lehtola et al. (2005). Nevertheless,

biofilm is slightly reduced because UV-treatment results in formation of reactive oxygen species, such as  $H_2O_2$  (Elasri and Miller, 1999).



**Figure 2. Evaluation of UV treatment, hypochlorite, and their combinations, regarding the inactivation of free-living (A) and biofilm-associated bacteria (B). Error bars indicate standard error of the mean. Asterisks indicate that the number of bacteria was below the detection limit of log 1 CFU/ml.**

Regarding hypochlorite treatment, three different concentrations were tested, i.e. 0.1, 1 and 5 ppm NaOCl (concentrations on active chlorine basis). Chlorination with 0.1 ppm NaOCl resulted in a 1.3 log reduction of the free-living bacteria after 72 h of treatment (Fig. 2A). Treatment with 1 ppm NaOCl almost completely eliminated the free-living bacteria, while 5 ppm hypochlorite resulted in complete inactivation after 24 h (data not shown). The combination of 0.1 ppm NaOCl and UV treatment resulted in a reduction of 3.3 log CFU/ml after 72h. Although no actual synergy was observed, a significant additive effect was clearly demonstrated. The combination of 1 ppm NaOCl and UV resulted in a total inactivation of free-living bacteria after 48h of treatment, which also points to an additive effect.

Hypochlorite treatment also resulted in a significant reduction of the biofilm-associated bacteria, but it did not completely remove the biofilm. NaOCl concentrations of 0.1 and 1 ppm resulted in reductions of 1.4 and 1.6 log CFU/cm<sup>2</sup>, respectively after 72h (Fig. 2B). The latter is considerably lower than the reduction in free-living bacteria achieved with 1 ppm hypochlorite treatment. This can be explained by the fact that biofilms provide a protected niche where microbes are more resistant to disinfectants (Lehtola et al., 2007). Consequently, higher doses of chemical disinfectants are required to effectively remove biofilms. When 0.1 ppm and 1 ppm NaOCl were combined with UV, a reduction of 1.4 and 1.7 log CFU/cm<sup>2</sup> was observed after 72h. These reductions were not significantly higher than treatment with hypochlorite alone, indicating that there was no significant additive effect regarding the biofilm treatment.

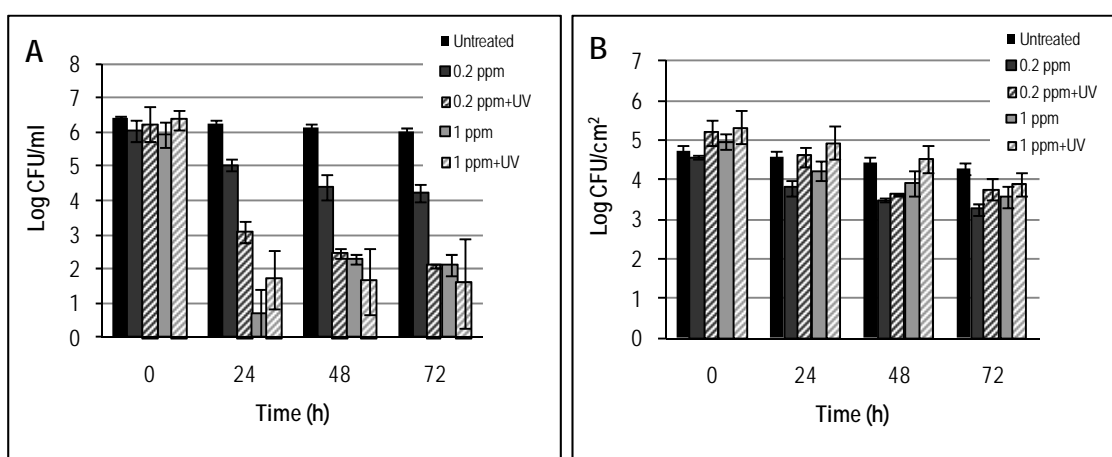
#### *Treatment with chlorine dioxide and UV*

Figure 3 shows the results of treatment with 0.2 ppm and 1 ppm  $ClO_2$  and their respective combinations with UV. Interestingly, treatment with 1 ppm  $ClO_2$  showed a significant rise in the number of free-living bacteria after 48 h. From thereon, the bacterial level remained at approximately 2.2 log CFU/ml. The same phenomenon was observed in three independent replicates of this experiment and was also reported by Murphy et al. (2008). This may be explained by the fact that some members of the mixed bacterial culture are completely inactivated, which enables a more resistant slow growing member to proliferate without

competition for nutrients. Alternatively,  $\text{ClO}_2$  treatment could induce a change in the physiological status of one of the inoculated strains, rendering it slightly resistant to  $\text{ClO}_2$  treatment.

Higher reductions in the number of free-living bacteria were obtained when 0.2 ppm and 1 ppm  $\text{ClO}_2$  were combined with UV. Reductions of 4.1 and 4.8 log CFU/ml were achieved after 72h, respectively. These data clearly point to significant additive effects of  $\text{ClO}_2$  and UV in the inactivation of free-living bacteria. However, it was also shown that chlorine dioxide is decomposed under the influence of UV light (Bergmann and Koparal, 2005). This was also observed in this study. The amount of  $\text{ClO}_2$  required to maintain a concentration of 1 ppm was nearly 60% higher when  $\text{ClO}_2$ -treatment was combined with UV (data not shown).

Regarding biofilm treatment, 0.2 and 1 ppm  $\text{ClO}_2$  resulted in significant reductions in the number of biofilm-associated bacteria: 1.3 and 1.4 log CFU/cm<sup>2</sup>, respectively after 72h. However, combination with UV did not reveal significant additive effects in biofilm removal.



**Figure 3. Evaluation of different concentrations of chlorine dioxide, and combinations thereof with UV, regarding the inactivation of free-living (A) and biofilm-associated bacteria (B). Error bars indicate standard error of the mean.**

#### *Treatment with hydrogen peroxide and UV*

As hydrogen peroxide is known to be less effective than chlorination, considerably higher concentrations (5, 50 and 100 ppm) were examined. Concentrations of 5 and 50 ppm  $\text{H}_2\text{O}_2$  resulted in a reduction of free-living bacteria of 0.8 and 3.6 log CFU/ml, respectively (Fig. 4). A concentration of 100 ppm completely removed the free-living bacteria after 24 h of treatment (data not shown).

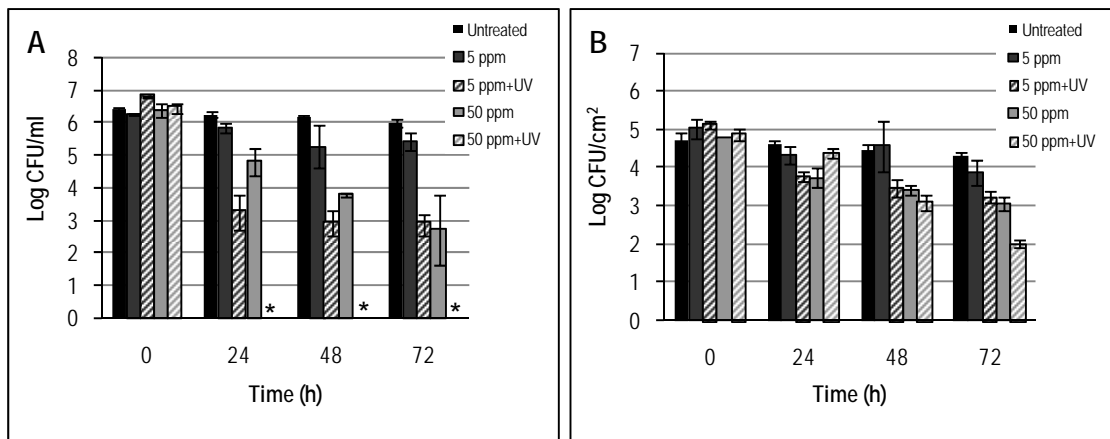
The removal of free-living bacteria was considerably more effective when hydrogen peroxide was combined with UV treatment. Combination of 5 ppm  $\text{H}_2\text{O}_2$  and UV resulted in a reduction of 3.9 log CFU/ml of free-living bacteria, demonstrating actual synergy of  $\text{H}_2\text{O}_2$  and UV on the inactivation of free-living bacteria. This is in agreement with the lab-scale experiments of Bianchini et al. (2002) that also demonstrated synergistic effects of hydrogen peroxide and UV in the inactivation of free-living bacteria. The increased efficacy is attributed to the increased production of hydrogen radicals in the presence of UV (Bianchini et al., 2002).

The combination of 50 ppm  $\text{H}_2\text{O}_2$  and UV completely removed the free-living bacteria. Since the separate treatment with either 50 ppm  $\text{H}_2\text{O}_2$  or UV only resulted in reductions of 3.6 and 3.0 log

CFU/ml, respectively after 72h, the total removal of free-living bacteria after combined treatment clearly points to a considerable additive effect. To unequivocally demonstrate synergy of 50 ppm H<sub>2</sub>O<sub>2</sub> and UV, experiments with higher initial inocula are required.

Regarding the evaluation of biofilm treatment, 5 ppm H<sub>2</sub>O<sub>2</sub> resulted in a reduction of 1.1 log CFU/cm<sup>2</sup> of biofilm-associated bacteria. Treatment with 50 and 100 ppm H<sub>2</sub>O<sub>2</sub> is more effective in biofilm treatment, with reductions of 1.7 and 3.3 log CFU/cm<sup>2</sup>, respectively. The combined treatment with UV and 5 ppm or 50 ppm H<sub>2</sub>O<sub>2</sub> resulted in a reduction of 1.9 and 2.9 log CFU/cm<sup>2</sup>, respectively, again pointing to a considerable additive effect.

Remarkably, both for inactivation of free-living bacteria and for biofilm treatment the combination of 5 ppm H<sub>2</sub>O<sub>2</sub>/UV is slightly more effective than treatment with 50 ppm H<sub>2</sub>O<sub>2</sub> alone. These data indicate that the required amount of hydrogen peroxide can be reduced 10-fold, when combined with UV. Furthermore, a preliminary analysis showed that the operational cost of treatment with 5ppm and UV is reduced with 30% compared to the treatment with 50 ppm hydrogen peroxide. These results clearly demonstrate considerable additive effects of UV and H<sub>2</sub>O<sub>2</sub> in biofilm treatment, and emphasize the benefit of combining chemical and physical disinfection techniques.



**Figure 4. Evaluation of different concentrations of hydrogen peroxide, and combinations thereof with UV, regarding the inactivation of free-living (A) and biofilm-associated bacteria (B). Error bars indicate standard error of the mean. Asterisks indicate that the number of bacteria was below the detection limit of log 1 CFU/ml.**

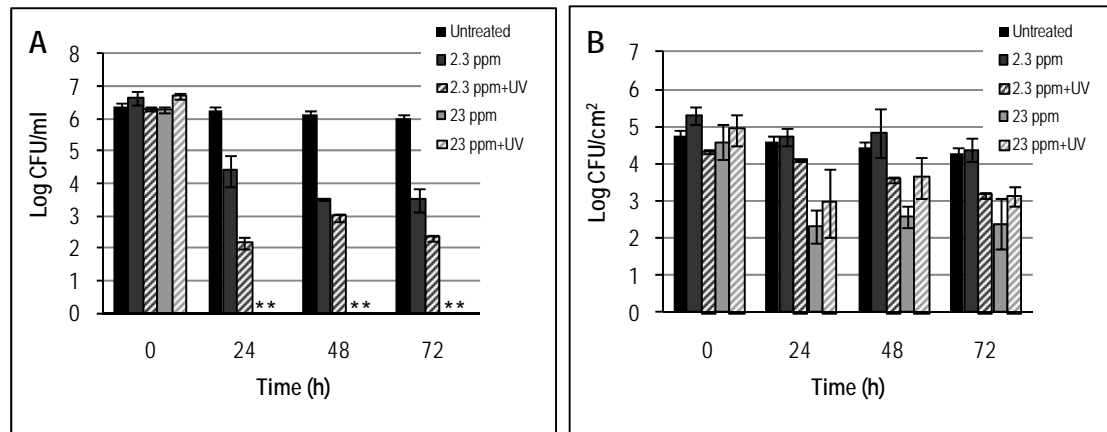
#### *Treatment with peracetic acid and UV*

Treatment with 2.3 ppm PAA resulted in a reduction of 3.2 log CFU/ml in free-living bacteria after 72 h of treatment (Fig. 5). Higher concentrations (23 and 230 ppm PAA), enabled complete removal of the free-living bacteria (Fig. 5 or data not shown). The combination of 2.3 ppm PAA and UV resulted in a 4.0 log CFU/ml after 72h, revealing a significant additive effect. Gori and Caretti (2008) measured an averaged 4.2 log CFU/ml reduction of *E. coli* after treatment of industrial wastewater with UV and 2 ppm PAA, which is comparable to our data.

In contrast to the data presented here, other studies demonstrated synergy of UV and PAA (Bianchini et al., 2002; Caretti and Lubello, 2003; Koivunen and Heinonen-Tanski, 2005). This can be explained by the fact that these studies involve only lab-scale experiments, indicating that pilot-scale experiments are highly valuable in evaluating disinfection techniques.

Regarding biofilm treatment, a 0.9 log CFU/cm<sup>2</sup> reduction of the biofilm-associated bacteria was obtained after 72h treatment with 2.3 ppm PAA, while PAA concentrations of 23 and 230 ppm

resulted in reductions of 2.2 and 4.4 log CFU/cm<sup>2</sup>, respectively after 72h. No significant additive effects were observed for the combination of PAA and UV in the removal of biofilm-associated bacteria.



**Figure 5. Evaluation of different concentrations of PAA, and combinations thereof with UV, regarding the inactivation of free-living (A) and biofilm-associated bacteria (B). Error bars indicate standard error of the mean. Asterisks indicate that the number of bacteria was below the detection limit of log 1 CFU/ml.**

## Conclusions

The main objective of this study was to explore possible additive or synergistic effects of suboptimal concentrations of the chemical disinfectants and UV disinfection on the treatment of biofilms and free-living bacteria (Table 1). Significant additive effects with respect to the inactivation of free-living bacteria were established when UV was combined with hypochlorite, chlorine dioxide, and PAA. For the combination of UV and hydrogen peroxide, actual synergy was observed regarding the inactivation of free-living bacteria for the lowest concentration tested in this study. For biofilm treatment, considerable additive effects were established. Notably, both for inactivation of free-living bacteria and for biofilm treatment the combination of 5 ppm H<sub>2</sub>O<sub>2</sub>/UV is slightly more effective than treatment with 50 ppm H<sub>2</sub>O<sub>2</sub> alone. These promising results demonstrate that combining hydrogen peroxide with UV can reduce the amount of chemicals 10-fold, which can ultimately lead to a more environmental-friendly water treatment strategy.

Although numerous studies already assessed the efficacy of disinfection techniques, most studies have a clear focus on either chlorine-based products or peroxides. However, it is very difficult to compare between the different disinfectants, because there is usually a big difference in the experimental set-ups. To our knowledge, this is the first study that evaluates hypochlorite, chlorine dioxide, peracetic acid and hydrogen peroxide, as well as their respective combinations with UV, in the same experimental set-up, which allows a good comparison between the different techniques. In addition, this is the first study that evaluates the combination of PAA with UV for the efficacy to remove biofilms.

Furthermore, in most studies, lab-scale experiments are used to assess disinfection and such experiments are mainly focused on monoculture biofilms. Compared to lab-scale experiments, the pilot-scale system used in this study provides a better simulation of actual industrial



processes in which water is prone to microbial contamination and is recirculated in a piping system because of the relevance of the scale and the use of mixed bacterial suspensions.

**Table 1. Overview of the efficacy of the chemical disinfectants, and their respective combinations with UV, regarding the inactivation of free-living and biofilm-associated bacteria.**

Technique	Concentration (ppm)	Reduction free-living bacteria(Log CFU/ml)		Reduction biofilm-associated bacteria(Log CFU/cm <sup>2</sup> )	
		Individual	+UV	Individual	+UV
UV		3.0	/	1.2	/
NaOCl	0.1	1.3	3.3 <sup>A</sup>	1.4	1.4
	1	6.3	6.7 <sup>A</sup>	1.6	1.7
	5	T.I.	n.d.	2.2	n.d.
ClO <sub>2</sub>	0.2	1.8	4.1 <sup>A</sup>	1.3	1.5
	1	3.8	4.8 <sup>A</sup>	1.4	1.4
PAA	2.3	3.2	4.0 <sup>A</sup>	0.9	1.2
	23	T.I.	T.I.	2.2	1.8
H <sub>2</sub> O <sub>2</sub>	5	0.8	3.9 <sup>S</sup>	1.1	1.9 <sup>A</sup>
	50	3.6	T.I. <sup>A,*</sup>	1.7	2.9 <sup>A</sup>
	100	T.I.	T.I.	3.3	3.2

Efficacy is expressed in log reduction of CFU/ml for free-living bacteria or log reduction of CFU/cm<sup>2</sup> for biofilm-associated bacteria after 72h of treatment. n.a.: not applicable; TI: total inactivation; n.d.: not determined; A: additive effect; S: synergistic effect; \*: further experiments with higher initial bacterial inocula may demonstrate a synergistic effect.

Indeed, multi-species biofilms are generally more resistant to disinfection compared to single-species biofilms (Elvers et al., 2002). This was confirmed by a previous study that clearly demonstrated that the results obtained from the pilot-scale system used in this study were in good agreement with case study experiments that were carried out in an industrial environment (Lambert et al., 2010). Indeed, our results differ somewhat from lab-scale experiments. For instance, some lab-scale studies reported synergistic effects in the inactivation of free-living and biofilm-associated bacteria for the combinations of PAA/UV, ClO<sub>2</sub>/UV, and NaClO/UV (e.g. Bianchini et al., 2002; Koivunen and Heinonen-Tanski, 2005; Dykstra et al., 2007; Rand et al., 2007). These data suggest that the disinfection efficacy may be overestimated in lab-scale experiments.

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